

Synanthedon pamphyla sp. n. from southern Turkey with a comparative analysis of mitochondrial DNA of related species (Sesiidae)

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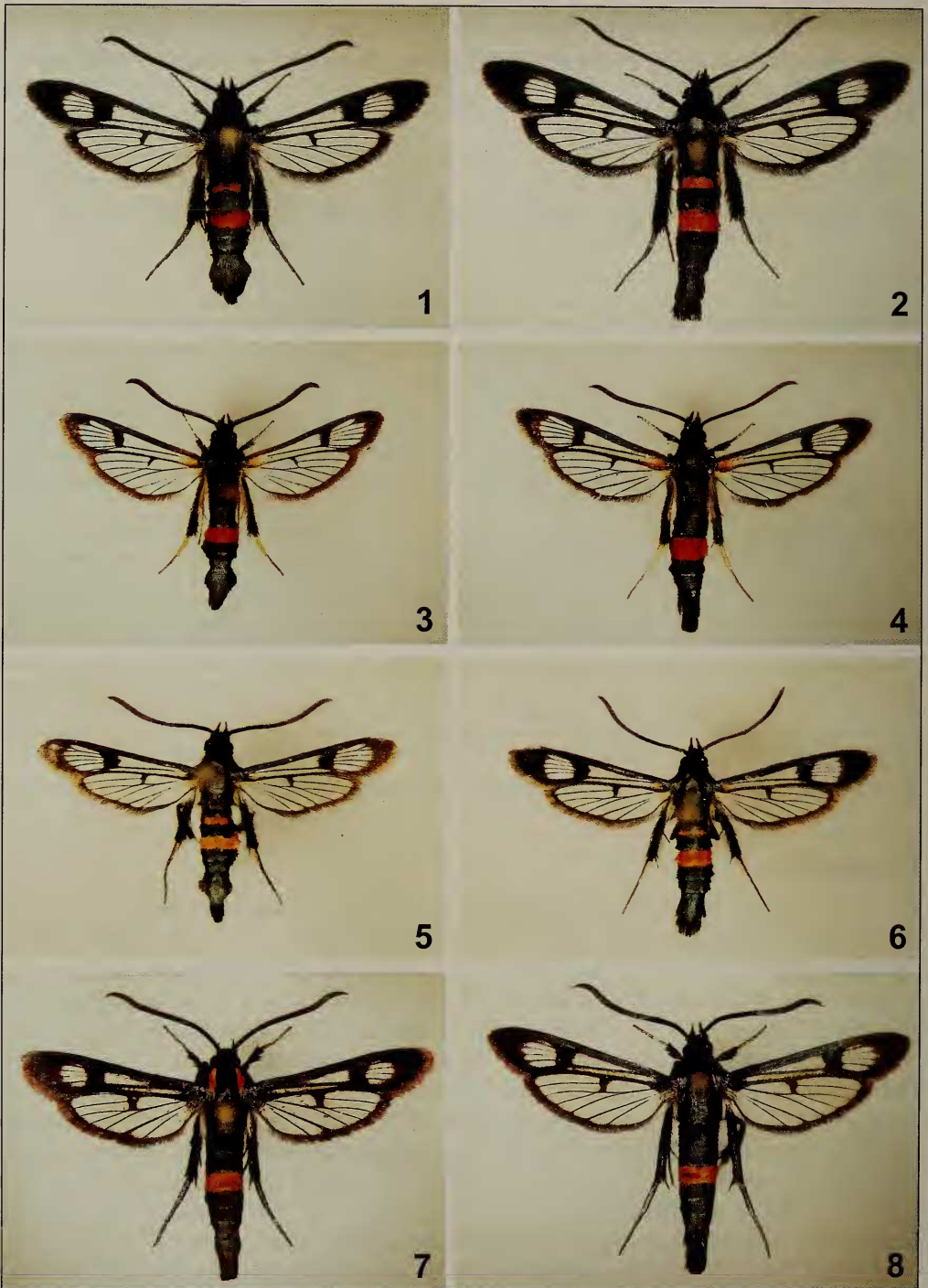
Abstract. *Synanthedon culiciformis* (Linnaeus, 1758) shows a circumpolar distribution and is recorded from most parts of the northern Palaearctic region and from North America. Its known sister species, *Synanthedon talischensis* (Bartel, 1906), however, is endemic to the Hyrcanian fauna and is found only in Talish south of the Caspian Sea. Here, another species, *Synanthedon pamphyla* sp. n., closely allied to *S. culiciformis* is described from southern Turkey. It is clearly separated from the known species by external characters, morphology of genitalia and bionomics. Further, sequences of two mitochondrial DNA regions of *S. culiciformis* and *S. pamphyla* sp. n. are analysed and compared to homologous sequences of the 'outgroup' species *Synanthedon spehiformis* ([Denis & Schiffermüller], 1775). This analysis suggests an isolation of *S. culiciformis* and *S. pamphyla* sp. n. for at least 300 000 years and implies that the latter species can be regarded as a Pleistocene relict.

Zusammenfassung. *Synanthedon culiciformis* (Linnaeus, 1758) ist eine circumpolar verbreitete Art, die in der gesamten nördlichen Palaearktis und in Nordamerika vorkommt. Ihre einzige bisher bekannte Schwesterart, *Synanthedon talischensis* (Bartel, 1906), ist dagegen ein Endemit der hyrcanischen Fauna und ist nur aus Talish südlich des Kaspischen Meeres bekannt. In dieser Arbeit wird eine weitere ebenfalls eng mit *S. culiciformis* verwandte Art, *Synanthedon pamphyla* sp. n., beschrieben, deren bisher bekannte Vorkommen auf die südliche Türkei beschränkt sind. Diese neue Art wird von den bekannten Taxa habituell, genitalmorphologisch und bionomisch abgegrenzt. Außerdem werden zwei mitochondriale DNS-Regionen von *S. culiciformis* und *S. pamphyla* sp. n. analysiert und mit homologen Sequenzen der "Außengruppen-Art" *Synanthedon spehiformis* ([Denis & Schiffermüller], 1775) verglichen. Diese Analysen deuten auf eine Isolation von *S. culiciformis* und *S. pamphyla* sp. n. seit mindestens 300 000 Jahren hin und lassen vermuten, daß es sich bei letzterer um ein Glazialrelikt handelt.

Key words. Lepidoptera, Sesiidae, new species, mitochondrial DNA, Turkey.

Introduction

In Asia Minor the family of clearwing moths (Sesiidae) is unusually rich in species. More than 100 species have been reported from Turkey alone (de Freina 1994; Spatenka *et al.* 1999; unpublished data). Like in the entire Mediterranean region or in Central Asia, the majority of species in Turkey belong to the rhizophagous genera *Bembecia* Hübner, [1819], *Chamaesphracia* Spuler, 1910, *Dipchasphecia* Capuse, 1973, and *Pyropteron* Newmann, 1832. Xylophagous genera, of which *Synanthedon* Hübner, [1819] is by far the most species-rich in the Palaearctic region, are poorly represented. Considering this, the finding of a large, undescribed *Synanthedon* species in Turkey is remarkable. Here, *Synanthedon pamphyla* sp. n., a species closely related to *Synanthedon culiciformis* (Linnaeus, 1758) and *Synanthedon talischensis* (Bartel, 1906), is described from the southern Toros Mts. From both related species *S. pamphyla* differs clearly in external characters and also in details of genitalia and bionomics. While *S. culiciformis* shows a wide circumpolar distribution with records from all over the northern Palaearctic and North America, *S. talischensis* is restricted to the Talish south of the Caspian Sea and is thought to be the sister species of *S. culiciformis* (Spatenka *et al.* 1999). *S. pamphyla* can be regarded as a remarkable addition to the *S. culiciformis* species group.



Figs. 1–8. *Synanthedon* species, all specimens in CAK. 1. *S. pamphyla*, male, holotype, Turkey. 2. *S. pamphyla*, female, paratype, Turkey. 3. *S. culiciformis*, male, Germany. 4. *S. culiciformis*, female, Germany. 5. *S. culiciformis*, male, Bulgaria. 6. *S. culiciformis*, male, Bulgaria. 7. *S. stomoxiformis amasina*, male, Turkey. 8. *S. stomoxiformis amasina*, male, Greece.

Up to now, proposed phylogenetic relationships within the family Sesiidae are solely based on morphological analyses. DNA sequence data that have been widely applied in Lepidoptera systematics in the past decade (reviewed in Caterino *et al.* 2000) have not been used for phylogenetic inference in Sesiidae. Nuclear genes, such as 18S rDNA, are one of the primary sources of molecular characters for relationships among more divergent groups (Hillis & Dixon 1991; Wiegmann *et al.* 2000). Mitochondrial genes have proven most useful for relatively recent divergences, especially of mid Tertiary and younger age and thus have been mainly used to reconstruct the phylogenetic relations of species within closely related taxa groups (Lopez *et al.* 1997). Except for a study on the pheromone binding protein (Willett 2000) published sequence data of Sesiidae are not available. In this work, two different mitochondrial DNA fragments were analysed to estimate the genetic divergence of the two closely related species, *S. pamphyla* sp. n. and *S. culiciformis*. The results presented here may stimulate further molecular research on the phylogeny of Sesiidae.

Material and Methods

DNA isolation, primers, PCR conditions. DNA was extracted from the abdomen of dried specimens using a rapid and simple salt-based method as described previously (Aljanabi & Martinez 1997). The ND1 fragment was amplified using forward primer (5'ACATGATCTGAGTTCAAACCGG) and reverse primer (5'GCTGGTTGATCTTCTAATTCTA) (Weller *et al.* 1994). It contains 576 nucleotides and comprises a part of the 16S ribosomal RNA gene, the tRNA-Leu gene and a portion of the first exon of the NADH dehydrogenase subunit I gene. The CO fragment consists of 586 nucleotides and contains the 3' end of the cytochrome oxidase subunit I gene, the leucine tRNA gene, and the 5' end of the cytochrome oxidase subunit II gene. It was amplified using the primers S2792 (5'ATACCTCGACGTTATTCAGA) and A3389 (5'TCATAAGTTCAATATCATTG) (Brown *et al.* 1994). For each specimen PCR fragments were obtained by two independent PCR reactions, cloned into pCR-XL-TOPO vector (Invitrogen) and sequenced by light cycler. PCR protocols were adopted from Weller *et al.* (1994) and Brown *et al.* (1999) respectively.

Specimens used for DNA analysis. *S. culiciformis*: ♂, Germany, Brandenburg, Neubrück near Gr. Köris, 2000, leg. Kallies & Garrevoet (Gen. prep. AK327, DNA-AK3) (CAK); ♂, Germania, Thüringen, Fischbachtal, 1991, leg. Eue (Gen. prep. AK328, DNA-AK4) (CAK); ♀, Russia, middle Volga region, 40 km NEN Uljanovsk, Yumanovka, forest, 1997, leg. Tumanova (DNA-AK11) (CAK); *S. pamphyla*: ♂, ♀ (paratypes, Gen. Prep. AK322, DNA-AK8; Gen. Prep. AK320, DNA-AK1) (CAK); *S. spheciformis*: ♂, Germany, Mecklenburg, Hüttelmoor near Rostock, 1996, leg. Ahrens (Gen. prep. AK338, DNA-AK5) (CAK).

Material examined for morphological comparison. *S. culiciformis*: Norway: ♀, Buskerud Fylke, Store Rödungen, Aal Kommune, 950 m, 1993, leg. Ockruck (CJG); France: ♀, Alpes, Tignes les Brév., 45°30'N 6°56'E, leg. Schmidt (CHR); Germany: ♂, Brandenburg, Oderberg, Pimpinellenberg, 1996, leg. Kallies (Gen. prep. AK321) (CAK); ♂, Brandenburg, Neubrück bei Gr. Köris, 2000, e.l., leg. Kallies & Garrevoet (Gen. prep. AK327) (CAK); ♂, Thüringen, Fischbachtal, 1991, leg. Eue (Gen. prep. AK328) (CAK); ♀, Mecklenburg, Grambow Moor near Schwerin, 1992, e.l. leg. Kallies (Gen. prep. AK323) (CAK); Austria: ♂ Lacken, 1942, leg. Reichl (Gen. prep. AK326) (CAK); Switzerland: ♀, Graubünden, Maloja pass, 1500m, 1991, leg. Riefenstahl (CHR); Russia: ♀, middle Volga region, 40 km NEN Uljanovsk, Yumanovka, forest, 1997, leg. Tumanova (CAK); ♀, Saratov distr., Polivanovka, 1929, lg. Richter (Gen. prep. AK355) (CAK); ♀, Transbaikal, Noworotnaja, Schilka river, 2000 m (CAK);

Bulgaria: 5♂, 2♀, Mitchurin, 1987 e.l., ex *Alnus*, leg. Lastuvka (CZL, CAK); USA: ♀, Maryland, Chestertown, 1999, leg. Mistera (CHR). *S. stomoxiformis*: Germany ♂, ♀, Werbach near Tauberbischofsheim, ex *Frangula*, 1991, leg. Kallies (CAK). *S. stomoxiformis amasina*: Turkey: ♂, Prov. Artvin, Kackar Dagi, Sarigöl-Yaylalar, 1500 m, 1995, leg. Kallies (CAK); Greece: ♂, Pelepones, Kardamili, 1999, leg. Lastuvka (CAK).

Abbreviations. ETA – external transparent area; PTA – posterior transparent area; ATA – anterior transparent area; CAK – collection A. Kallies; CHR – collection H. Riefenstahl, Hamburg, Germany; CJG – collection J. Gelbrecht, Königs Wusterhausen, Germany; CZL – collection Z. Lastuvka, Brno, Czech Republic.

Taxonomic part

Synanthedon pamphyla sp. n.

Material. Holotype ♂ ‘Turkey S, Toros Mts, ca. 25 km NW Alanya, Güzelbag, ca. 500 m, larva 30.X.2001, ex *Alnus orientalis*, 9–25.III.2002 e.l., leg. A. Kallies, A. Musolff & Th. Drechsel’ (CAK, the holotype will be deposited in the Museum für Naturkunde Berlin, Germany). Paratypes: 11♂, 10♀, same dates as holotype (CAK) (of two males and one female genitalia examined, gen. prep. AK320, AK322, AK325); ♀, same locality data as holotype, 12.IV.2001 on leaf of *Alnus orientalis*, leg. Th. Drechsel (CAK); ♂, 4♀, Turkey S, Toros Mts, ca. 10 km E Alanya, Dimcay river valley, ca. 200 m, larva, early IX.2001, ex *Alnus orientalis*, 9–25.III.2002 e.l., leg. A. Kallies, A. Musolff & Th. Drechsel (CAK); 3♀, Turkey, between Ödemis and Salihli, northern slope of Boz Dagi, 1000 m, 38°22’N 28°07’E, 19.V.1983, leg. E. Hüttinger (CZL); ♀, Turkey, Prov. Izmir, 3 km N Bozdag (Birgi-Salihli), 900 m, 38°22’N 27°58’E, 22.V.1981, leg. H. & R. Rausch, F. Ressler (CZL).

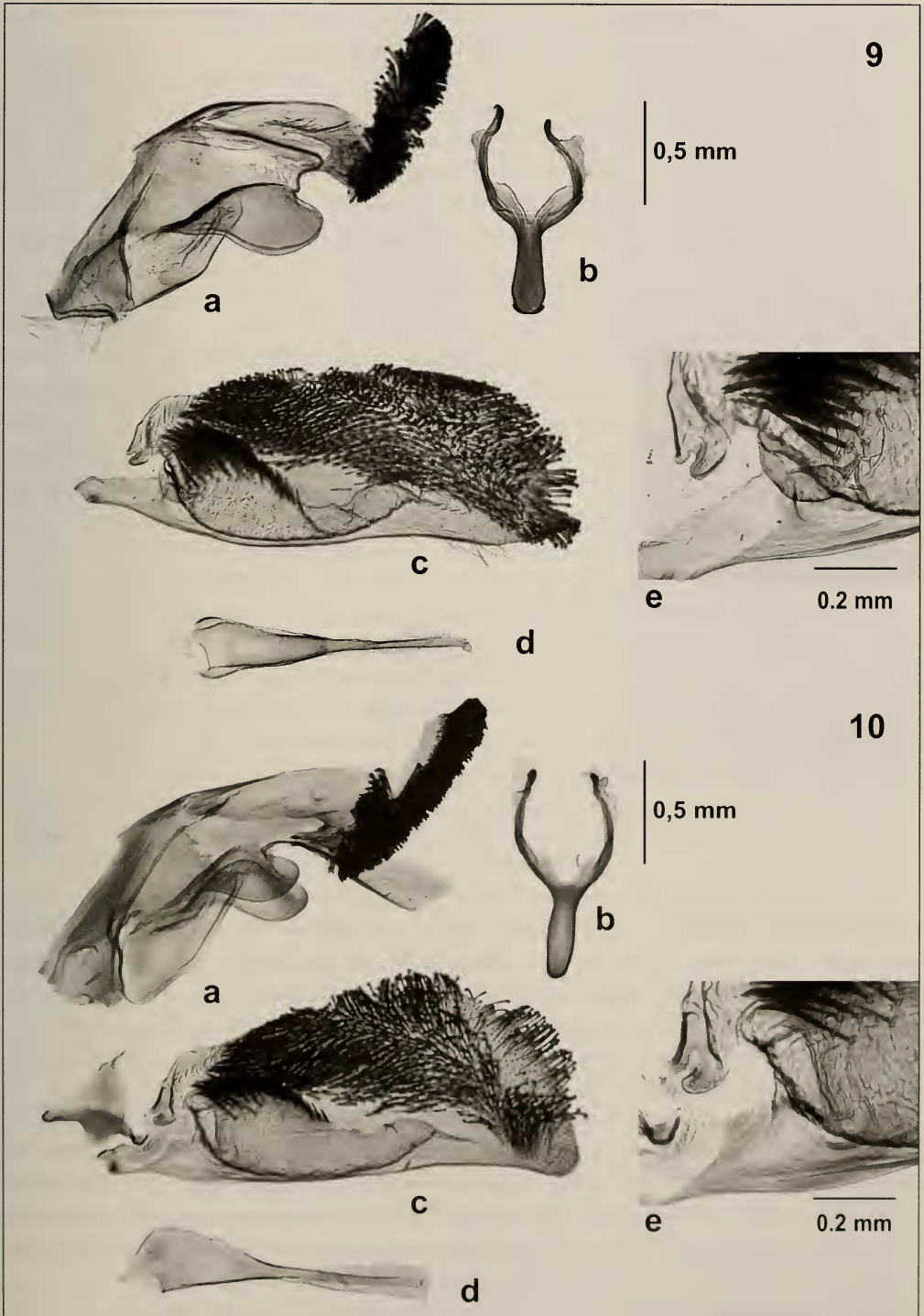
Male (holotype, Fig. 1). Alar expanse 26.0–28.0 mm, body length 14.0–16.0 mm, forewing length 11.5–12.5 mm, antenna 8.3–8.6 mm.

Head. black almost throughout; frons laterally bright white; pericephalic scales laterally orange. Thorax: black; ventrally with a large orange-red spot; mesothorax with white hairlike scales dorso-laterally. Legs: entirely black. Abdomen: black; tergite 2 dorsally with a red orange band along posterior margin, laterally with orange-red spots; segment 4 with a broad orange-red, ventrally open band; anal tuft completely black. Forewing: discal spot broad, with a narrow and short projection into the ATA; PTA well-developed, reaching under discal spot; ETA round, relatively small, maximally about 1.5× broader than discal spot; apical area broad and black, cell between R4 and R5 opaque; veins, costal and anal margins dorsally black, the latter with a few individual red scales near base; ventrally cubitus, costal and anal margins covered with orange scales; fringe black. Hindwing: with relatively broad discal spot which reaches M3; ventrally basal portion of the costal margin orange.

Female (paratype, Fig. 2). Very similar to male but larger (alar expanse 27.0–30.5 mm, one female with 24.0 mm), ETA somewhat smaller; ATA with some black scales in distal portion; outer margin of hindwing broader.

Genitalia (Figs 10, 12). Very similar to that of *S. culiciformis* (diagnosis below).

Diagnosis. This new species is closely related and similar to *Synanthedon culiciformis* and *S. talischensis*. From both species, however, it can be easily separated by external characters. *S. pamphyla* differs from *S. culiciformis* by the broader discal spot and the smaller ETA of the forewing (ETA maximally 1.5× broad than discal spot; 3× broader in *S. culiciformis*), by the broader apical area (along R3 about as broad as ETA; 1/3 to 1/2× as broad in *S. culiciformis*), by the opaque cell between Cu1 and Cu2 (transparent in *S. culiciformis*), by the shape of the PTA (just reaching the discal spot; reaching beyond in *S. culiciformis*), by the equally broad discal spot of the hindwing



Figs. 9-10. Male genitalia. a - uncus-tegumen, b - saccus, c - right valva, d - aedeagus (scale bar 0.5 mm), e - basal part of the crista sacculi (scale bar 0.2 mm). 9. *Synanthedon culiciformis*, Germany (gen. prep. AK327, CAK). 10. *Synanthedon pamphyla* sp. n., Turkey (gen. prep. AK325, CAK).

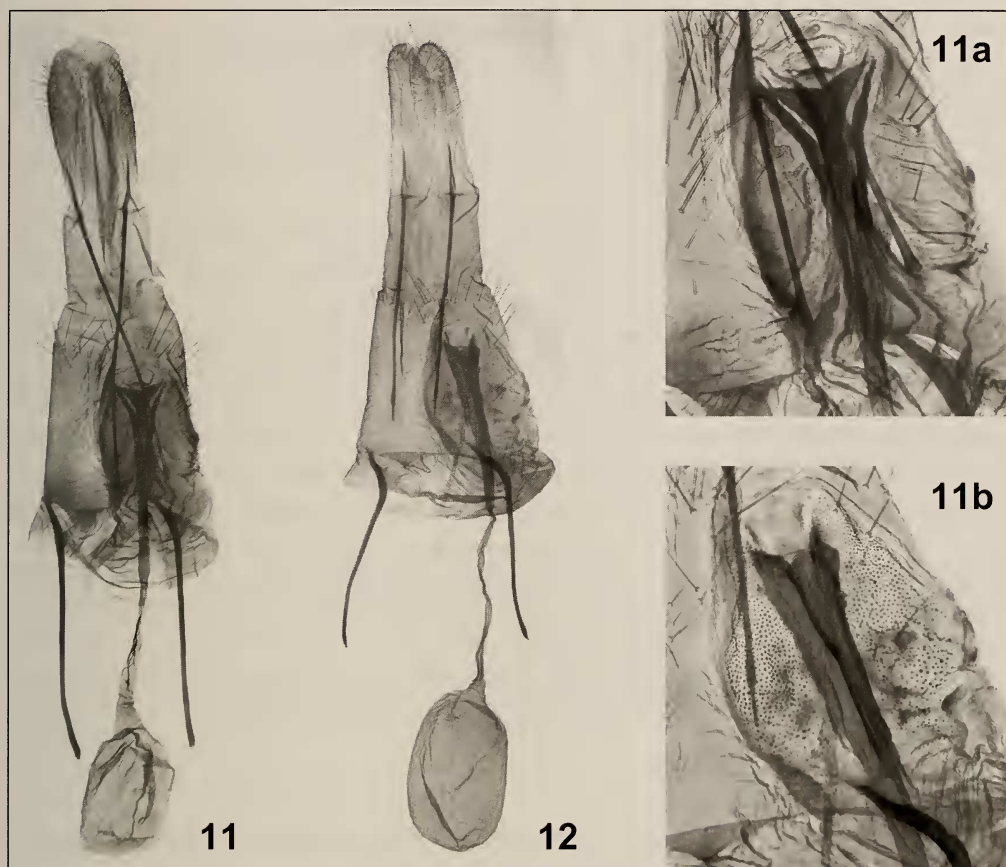
(narrower and pointed towards M2 in *S. culiciformis*), by the almost complete absence of red scales at the forewing base (present and very pronounced in *S. culiciformis*), by the black labial palps (ventrally red in *S. culiciformis*), by the completely black legs (tarsomers yellow in *S. culiciformis*), by the color of the abdomen (in *S. culiciformis* tergite 2 only occasionally red, red ring of segment 4 ventrally closed), and by the color of the ventral side of the forewing (orange-red scaling extending into the apical area in *S. culiciformis*). Further, *S. pamphyla* is conspicuously larger than *S. culiciformis* (alar expanses in males of *S. culiciformis* only 20–26 mm, in females 23–28 mm). From *S. talischensis* the new species differs by completely black antennae (distally white in *S. talischensis*), by the black labial palps (ventrally red in *S. talischensis*), and by the broad discal spot of the hindwing (small in *S. talischensis*).

Consistent differences between *S. pamphyla* and *S. culiciformis* are also found in the morphology of the genitalia (Figs. 9–12). In male *S. pamphyla*, the valva is broad and arched, the distal end of the crista sacculi is relatively close to the ventral margin of the valva (valva narrow and straight, crista sacculi stronger bent with distal end more close to the setae field in *S. culiciformis*), the small 'secondary' crista connecting the proximal portion of the crista sacculi with the surface of the valva (Figs. 9e, 10e) is absent while it is present in *S. culiciformis*, the crista medialis of the gnathos is distally somewhat longer and more pronounced than in *S. culiciformis*, and the ventral margin of the crista medialis is simple while it is cloven in *S. culiciformis*. In female genitalia (Figs. 11, 12), *S. pamphyla* displays short apophyses anteriores which do not reach the corpus bursae (longer, reaching the corpus bursae in *S. culiciformis*) and the deepening in which the ostium and the proximal part of the ductus bursae is situated is covered with small well-sclerotized hooks (nearly absent in *S. culiciformis*). Furthermore, the ostium bursae is narrower, not conspicuously funnel-shaped as it is in *S. culiciformis*, and shows a small roundish distal plate ventrally which is absent in *S. culiciformis*. Since there was no material of *S. talischensis* available for detailed genitalia examination potential differences could not be investigated.

Besides the two species compared above, *S. pamphyla* is also similar to *S. stomoxiformis* (Hübner, 1790). Externally the latter can be separated by the black abdominal segment 2, the partially red tegulae, the laterally orange edged anal tuft, the completely black frons, and the ventrally black thorax. Moreover, *S. stomoxiformis* displays a completely different morphology of the genitalia (cf. Spatenka *et al.* 1999).

Variability. Except for size, *S. pamphyla* is almost invariable in terms of external appearance. In some regions (Bulgaria, southern Russia) specimens of *S. culiciformis* with relatively small transparent areas and broad discal spots can be found. These specimens frequently display a red ring on abdominal segment 2. However, additional characters such as the red labial palps, yellowish tarsi and ventrally closed red abdominal ring are consistent and distinguish these populations from *S. pamphyla*. More importantly, characters of male and female genitalia used to differentiate between *S. culiciformis* and *S. pamphyla* did not show any variation.

Distribution. To date *S. pamphyla* is known from the southern part of Turkey (Provinces of Antalya and Izmir), however, this species can probably be found associated with its hostplant along the Mediterranean coast of Turkey.



Figs. 11–12. Female genitalia, ventral view (scale bar 0.5 mm), **a** – ostium and proximal part of ductus bursae (scale bar 0.2 mm). **11.** *Synanthedon culiciformis*, Germany (gen. prep. AK323, CAK). **12.** *Synanthedon pamphyla* sp. n., Turkey (gen. prep. AK320, CAK).

Records of *S. culiciformis* from Turkey (Spatenka *et al.* 1999) may at least in part relate to specimens of *S. pamphyla* of which one was figured previously (Lastuvka & Lastuvka 1995: pl. 3 fig. 10, misidentified as *S. culiciformis*). The presence of *S. culiciformis* in Turkey needs verification. While this species may occur in the northern part of Turkey it is very unlikely to be found in the southern provinces. Specimens of *S. culiciformis* (Figs 6–7) from Micurin, Bulgaria, are the records which are geographically closest to the known range of *S. pamphyla*.

Bionomics. The hostplant of the new species is *Alnus orientalis* Decne (Betulaceae). This tree grows along rivers and streams on the southern slopes of the Toros Mts often accompanied by *Nerium* (Apocynaceae), *Platanus* (Platanaceae) or *Vitis* (Vitaceae). Typically *S. pamphyla* inhabits the main shoot of young trees from 1 to 6 cm diameter where it can be found from close to the root up to 4 m high in the tree. Infested trees usually can be recognised easily by a conspicuous swelling which is often accompanied by a patch of dead and dry bark. In autumn the full grown larva is found in a tunnel which leads from the swelling 8 to 15 cm upwards within the

wood of the stem. Pupation takes place in early spring head-down in a cocoon made from narrow wooden chips which is tightly attached to the tunnel's inner surface. The emerging hole is closed by fibrous wood chips which are pressed out of the hole by the larva. Occasionally, larvae can be found associated with injuries of the stem. Several larvae were found in the remnants of an older *Alnus* tree which was cut down a year before.

The known localities of *S. pamphyla* are within the Eumediterranean to Mesomediterranean climate zone on the slopes of the Toros mountain range mostly between 200 and 500 m. The average temperature in January is 5–9 °C and in summer between 25 and 27 °C. Temperatures below zero are rare in this zone. Under laboratory conditions branches which contained larvae, were stored from early December to early March in a humid environment at about 5 to 15 °C. After placing the branches at room temperature adults emerged within 3 to 19 days. From this it is assumed that under natural conditions adults can also be found in early spring probably from March to April. So far, however, only one adult specimen, a somewhat worn female, was observed at the type locality by Mr. Thomas Drechsel on the 12th of April. Some additional specimens which are also part of the type series have been collected at an altitude of 900–1000 m on the slopes of the Boz Mts near Izmir. These specimens, all females, were collected at the end of May.

Derivatio nominis. The species name derives from the ancient kingdom of Pamphylia which was situated east of Antalya about 3000 years ago.

Genetic analysis

Analysis of sequence divergence. To estimate the genetic distance between *S. culiciformis* and *S. pamphyla* and to initiate molecular characterisation of Sesiidae in systematics, the sequence of two mitochondrial DNA fragments, ND1 and CO, was analysed. As a prelude to interspecific analysis, the intraspecific variation within the ND1 and CO fragments was determined. While specimens of individual populations (as determined for the ND1 fragment in *S. culiciformis* from Brandenburg and *S. pamphyla* from the type locality) in general did not show sequence variation, a series of Thymidines (positions 31 to 39 of the fragment) within the 5' end of ND1 fragment was found to be of variable length even among specimens of the same population. Since this region could not be reliably aligned it was omitted from analysis as was suggested by Brower (1994). Under these conditions, in the ND1 fragment seven positions (1.23%) and in the CO fragment five positions (0.85%) were consistently different between specimens of *S. culiciformis* from central Europe and specimens of *S. pamphyla* from the type locality in southern Anatolia. Of these changes only one, within the ND1 fragment, was modified by transversion, all other changes were transitions. The comparison of the sequences generated from a specimen of *S. culiciformis* from the Volga region of southern Russia and specimens of *S. pamphyla* indicated a higher degree of similarity. In this approach only 0.7% sequence divergence (four positions) for the ND1 fragment or 0.85% (five positions) for the CO fragment were found between the two taxa. However, while among specimens of *S. culiciformis* from Brandenburg and Thuringia only one position was found to be targeted by substitution, the mean divergence between these specimens and the specimen from southern

Tab 1. Uncorrected pairwise distances (below diagonal) and transition / transversion ratio (above diagonal). GB – Germany, Brandenburg; GT – Germany, Thuringia; RV – Russia, Volga region

	1	2	3	4	5
1 <i>S. spheciiformis</i>	–	3.4	3.4	3.3	3.0
2 <i>S. culiciformis</i> ‘GB’	0.0925	–	–	12.0	12.0
3 <i>S. culiciformis</i> ‘GT’	0.0925	0.0009	–	13.0	12.0
4 <i>S. culiciformis</i> ‘RV’	0.0900	0.0104	0.0113	–	–
5 <i>S. pamphyla</i>	0.0900	0.0104	0.0104	0.0078	–

Russia was as high as 0.97% indicating a genetic distance almost as high as the distance between central European *S. culiciformis* and *S. pamphyla*. In fact, a phylogenetic tree based on the mitochondrial sequence data generated in this study would group the specimen from the Volga region and *S. pamphyla* as sister taxa (not shown).

As an ‘outgroup’ species *Synanthedon spheciiformis* ([Denis & Schiffermüller], 1775) was used in this study. The ND1 fragment of this species shows a divergence of about 9% to both *S. pamphyla* and *S. culiciformis* with several indels (gaps and insertions) in the 5’ noncoding part of the fragment. Table 1 shows the uncorrected pairwise distances for all haplotypes as calculated from the ND1 and CO fragment (since for *S. spheciiformis* a CO fragment could not be generated, distances and ratios for this species relate only to the ND1 fragment) and the ratio of transitions versus transversions. All sequences are available at DDBJ/EMBL/GenBank, Accession Nos AY304162-70.

Applying a ‘molecular clock’. Brower (1994) has proposed a molecular clock for arthropod mitochondrial DNA and assumed a constant pairwise mutation rate of about 2.3% per million years. Applying this molecular clock the corresponding age of the separation of *S. culiciformis* and *S. pamphyla* is estimated to be 300 000 to 500 000 years. The divergences found in comparison of *S. spheciiformis* to the species of the *S. culiciformis* group suggests a separation nearly 5 million years ago.

Discussion

The tribe Synanthedonini comprises an extensive group of clearwing moths which is found in all zoogeographic regions. There are several genera recognized in the tribe of which the genus *Synanthedon* (sensu auctorum) which includes mainly xylophagous species, is by far the most species rich. However, in the Palearctic region rhizophagous genera, i.e. *Dipchasphecia*, *Bembecia*, *Pyropteron*, and *Chamaesphecia*, account for the bulk of Synanthedonini species. Most of the members of these genera can be placed into species groups partly containing large numbers of closely related species which are often found only in small ranges suggesting relatively recent radiations within the Palearctic region. While the rhizophagous genera are mainly well defined monophyletic groups, the genus *Synanthedon* in the present concept is likely to be paraphyletic (Lastuvka 1992a, b; unpublished data).

In most cases, Palearctic *Synanthedon* species can be differentiated clearly by external and genitalic characters and often appear only distantly related, a view which is supported by the finding of this study (i.e. the large genetic distance of *S. spheciiformis* and the species of the *S. culiciformis* group). There are only few and usually

small species groups such as the *S. formicaeformis* (Esper, 1783), *S. tipuliformis* (Clerck, 1759), and *S. vespiformis* (Linnaeus, 1761) groups in the Palearctic region and the formation of local endemics, such as *S. geranii* Kallies, 1997 from Greece, is rather unusual, suggesting that recent species radiation in Palearctic *Synanthedon* is not as common as in rhizophagous *Synanthedonini*.

The Holarctic *Synanthedon culiciformis*, the south Anatolian *S. pamphyla* and the Hyrcanian (south-west Caspian) *S. talischensis* are closely related but strictly allopatric species which share broad morphological, bionomical, and genetic similarities (as shown for two species). While *Synanthedon culiciformis* shows a circumpolar distribution with records from all over the northern Palearctic and North America, *S. talischensis* and *S. pamphyla* are restricted to the Talish south of the Caspian Sea and to the southern Toros Mts of Anatolia, respectively. To answer the question of how long populations of these three species have been separated and the gene flow between has been disrupted, it is appropriate to consider the history of climate changes in Asia Minor. Like in the entire northern hemisphere, the climate of Anatolia has been strongly influenced by the glacial ages. Periods of milder to warm climates alternated with periods of colder climates which triggered the spreading of arctic and boreal flora and fauna towards the south. During the past 1.7 million years at least 17 glacial-interglacial oscillations occurred in the Mediterranean region (Bertolani-Marchetti 1985). Glacial periods, such as the Würm glacial, caused the progression of the subarctic region deep into southern Europe and Asia Minor. During these times the average temperature in present Anatolia was about 8 to 10 °C lower than today and a maximal extension of the distribution of arctic and boreal species such as *Synanthedon culiciformis* can be supposed. At the same time, however, Eumediterranean vegetation was well-established in the lower parts of the southern Toros Mts (Wagner 1995). It can be assumed that in line with the glacial oscillations a geographic separation and lasting isolation of at least two different populations of the *Synanthedon culiciformis* group took place. Genetic analysis indicates that speciation occurred 300 000 to 500 000 years ago suggesting an isolation during early glaciation events. Later, regression of the arcto-boreal vegetation led to a geographic isolation and an interruption of a potential gene flow between the isolated ancestor populations of *Synanthedon pamphyla* and *Synanthedon talischensis* on one side and *Synanthedon culiciformis* on the other side.

Refugia, such as the Hyrcanian and the Tauro-Mediterranean regions, were essential for the survival of flora and fauna during the glacial periods of Pleistocene. These regions contain many endemic species (Wagner 1995) and *Synanthedon pamphyla* may represent another example of endemism in the southern Toros Mts.

Although there is a general trend for reproductive isolation to increase with genetic divergence this relationship is hardly predictive for identifying new species. A broad variation in genetic distance between sibling species has been found in different studies ranging from undetectable to more than 13% (reviewed in Ferguson 2002). Several studies indicate that the mitochondrial DNA evolves at a similar rate in a wide range of organisms (Brower 1994; Avise *et al.* 1998) but the duration of speciation varies widely from several thousand years, as shown for fishes, and several million years, in mammals and other vertebrates (reviewed in Avise *et al.* 1998).

Analysis of mitochondrial sequences carried out in this study revealed a degree of divergence between *Synanthedon pamphyla* and *Synanthedon culiciformis* of

0.78–1.04% consistent with rates of between 0.19% and 5% found for haplotypes within species groups or for sibling species in other studies on Lepidoptera (Caterino & Sperling 1998; Brown *et al.* 1999; Blum *et al.* 2003). This result and the high ratio of transitions to transversions support a very close relationship of both species, in agreement with the hypothesis of a speciation during the Pleistocene. Further, in this study surprisingly clear sequence differences between populations of *S. culiciformis* from the ‘western’ (central European) and the ‘south-eastern’ part (southern Russia) of the range were found. In fact, the distance between the Russian *S. culiciformis* compared to central European *culiciformis* was higher than the distance between Russian *S. culiciformis* and *S. pamphyla* (Tab. 1). This result as well as external characters of *S. culiciformis* from southern Russia and Bulgaria (such as the broad discal spot and the small transparent areas of the forewing) which distinguish these populations from central European *S. culiciformis* suggest that the south-eastern clade of *S. culiciformis* is well separated from the main part of the species range and may represent the sister taxon of *S. pamphyla* leaving *S. culiciformis* in the present concept paraphyletic. More material of *S. culiciformis* from various parts of its range especially from Bulgaria and southern Russia as well as material of *S. talischensis* and its detailed examination both on morphological and molecular level is necessary to decide whether a further taxonomic differentiation of the *S. culiciformis* group is appropriate.

The higher degree of sequence similarity between the south Russian *S. culiciformis* and *S. pamphyla* suggests that *S. pamphyla* has evolved from an isolated population of the ‘south-eastern clade’ of *S. culiciformis*. Interestingly, the specimens from Bulgaria just like *S. pamphyla* and *S. talischensis* were bred from *Alnus*, which is utilized by *S. culiciformis* only occasionally as a host plant. It could be speculated that speciation in the *S. culiciformis* group was promoted not only by geographic isolation but also by host plant switch.

As demonstrated by Ferguson (2002) genetic distance itself is not sufficient for species identification since several additional factors, e.g. degree of sympatry and geographical range, have a strong effect on the genetic distance measured. Accordingly, in this work, genetic distance was not used to separate *S. pamphyla* from *S. culiciformis*, rather mitochondrial sequence analysis was applied to reconstruct speciation in a temporal and geographical frame.

As an ‘outgroup’ species *S. sphecoformis* was used in this study. This species is well separated from the species of the *S. culiciformis* group. Mitochondrial DNA divergences found in comparison of *S. sphecoformis* to the species of the *S. culiciformis* group suggest a much earlier separation, nearly 5 million years ago. This is in line with morphological data indicating a more distant relationship between *S. sphecoformis* and the *S. culiciformis* species group.

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